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June 1946 Supplement to AIC-106

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Agricultural Research Administration
U. S. Department of Agriculture

Effect of Subtilin on Surface Tension

It has been shown by Anderson *et al* (1) that subtilin in a dilution of 1:2000, either in water or in 85 percent ethyl alcohol, causes an immediate reduction in surface tension. They point out that this concentration is within the range in which it exerts antibiotic activity.

Antibiotic Effects of Subtilin

Subtilin has now been shown to be active against a considerable number of bacteria, many of which are pathogenic to animals, including man. Salle and Jann (2) tested subtilin against 52 organisms (including in some cases different strains of the same species). Of these, 21 (all gram negative) were not affected by subtilin in a dilution of 1:1000. The remaining 31 were inhibited by subtilin in dilutions of 1:1000 to 1:1,000,000. All but 3 of these were gram positive. Table 4 gives a partial list of these organisms.

The test method used for most of these organisms is described as follows by the above authors: "To 20 cc. of melted and cooled agar was added 0.1 cc. of a 24-hour nutrient broth culture of the test organism. The inoculated agar was poured into a sterile Petri dish, thoroughly mixed, and allowed to harden. The effect of the antibiotic was shown by (1) pipetting 0.1 cc. of subtilin directly into a cup 10 mm. in diameter in the agar or (2) placing on top of the agar a 10 mm. disc of filter paper (Whatman No. 2) previously soaked in subtilin solution. The inhibitory action of the subtilin manifested itself as a clear zone around the agar cup or disc of filter paper." *N. gonorrhoeae* was tested similarly except that the plate was inoculated by streaking it with the organism. *M. tuberculosis* was inoculated into tubes of medium containing varying amounts of subtilin.

Salle and Jann (2) have also tested subtilin against 10 actinomyces and fungi, of which 4 were susceptible to it. These were tested by streaking the organism over the surface of plate and placing a filter paper disc soaked in a 1:1000 dilution of subtilin on the agar.

Since subtilin inhibited certain pathogenic bacteria *in vitro*, the next step was to determine its activity *in vivo*. In order to do this some information was necessary as to the effect of subtilin on possible hosts. The toxicity of subtilin to chick embryo tissue grown in culture was determined by Salle and Jann (3) and found to be only 5 percent as great as its toxicity against *Staph. aureus*.

Wong and Anderson (5) showed that guinea pigs could be sensitized to subtilin by large doses. Animals receiving three sensitizing doses of 60 mgm. (in one case 30 mgm.) per kg. body weight died in typical anaphylaxis after shocking doses of 5 and 10 mgm.

Table 4 - Partial List of Organisms Tested for Susceptibility to Subtilin

(Abstracted from Salle and Jann (2))

Organisms Susceptible to Subtilin	Organisms not Susceptible to Subtilin
Bacillus anthracis	Brucella abortus
" megatherium	" suis
Corynebacterium diptheriae	Eberthella typhosa
Diplococcus pneumoniae I (parent strain)	Escherichia coli
" " II (sulfa resistant)	Salmonella paratyphi
Mycobacterium phlei	" schottmuelleri
" smegmatis	Serratia marcescens
" tuberculosis	Shigella alkalescens
Sarcina lutea	" dysenteriae
" ureae	" paradysenteriae
Staphylococcus aureus	Vibrio comma
" citreus	
Streptococcus faecalis	
" lactis	
" pyogenes (hemolytic)	
Neisseria catarrhalis	
" gonorrhoeae	

Data on the action of subtilin against pathogens *in vivo* are as yet very meagre. Salle and Jann (4), however, have shown that subtilin protected mice against intraperitoneal injections of Type III pneumococcus. Protection was afforded by intraperitoneal injections of 0.1 mg. of subtilin repeated seven or eight times during a 48-hour period following inoculation.

Anderson *et al* (1) tested subtilin against four animal parasites. Most encouraging results were with *Endamoeba histolytica*, which was killed *in vitro* by subtilin in a dilution of 1:400,000. One monkey was cleared of this organism for three weeks after 1.0 gram per kg. total oral doses in 10 days. Other organisms were affected as follows:

Trypanosoma equiperdum: Immediate cytolysis caused by subtilin in a 1:2000 dilution. Survival of trypanosome-infected mice was not prolonged by doses of 80 to 160 mg./kg. given intraperitoneally.

Leishmania donovani and *Ascaris suis*: Subtilin was not active *in vitro* or *in vivo*.

Preparation of Sterile Solutions of Subtilin

In order to test an antibiotic against a pathogen *in vitro*, it is usually necessary to prepare a sterile solution of the antibiotic. Since certain difficulties have been experienced in preparation of sterile subtilin solutions, a procedure for this is outlined.

A solution containing approximately one percent subtilin is prepared by shaking the dry subtilin with distilled water. This operation should be carried out in a small vessel, such as a stoppered test tube or a glass-stoppered graduate, whose volume is not over twice the volume of the solution. This will permit all of the dry powder to be washed off into the solution, and it will prevent formation of an excessive amount of foam during the shaking. Shaking should be done on a shaking machine or at intervals by hand during a period of at least half an hour. Lot No. 111 contains a small amount of material which will not dissolve in water.

After the solution is prepared, it is brought to a pH of 2.5 to 3.0 by the addition of 5 percent HCl. It is then steamed in an autoclave at about 100° C. for 10 minutes. The container in which it is steamed should preferably be pressure-sterilized previously, since steaming will not sterilize the dry portions of the container.

When the acidified subtilin is added to the nutrient medium in which the test organism is to be inoculated, it may significantly alter the pH of any medium which is not sufficiently buffered. This can, of course, be prevented by adding sterile NaOH to the medium in an amount equivalent to the HCl which is added with the subtilin. If a large number of tests are being made, it is more convenient to neutralize the subtilin solution before it is added to the medium. This can be done with sterile 1 normal NaOH (equivalent in amount to the previously added HCl). Care must be exercised in order to avoid a pH greater than 7.5 and possible loss of activity. If this is done, the resulting NaCl reduces the solubility of the subtilin, causing it to form a colloidal precipitate. This has not been observed to flocculate, however, and the suspension can be added to the medium without loss of subtilin activity.

It would, of course, be simpler to filter the subtilin solution. It has been found, however, that a Seitz filter removes most of the activity and that even a sintered glass UF filter removes part of the activity from a one percent solution.

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